Timing and rate of genome variation in triticale following allopolyploidization

Xue-Feng Ma and J. Perry Gustafson

Abstract: The timing and rate of genomic variation induced by allopolyploidization in the intergeneric wheat–rye (*Triticum* spp. – *Secale cereale* L.) hybrid triticale (× *Triticosecale* Wittmack) was studied using amplified fragment length polymorphism (AFLP) analyses with 2 sets of primers, *Eco*RI–*Mse*I (E–M) and *Pst*I–*Mse*I (P–M), which primarily amplify repetitive and low-copy sequences, respectively. The results showed that allopolyploidization induced genome sequence variation in triticale and that a great degree of the genome variation occurred immediately following wide hybridization. Specifically, about 46.3% and 36.2% of the wheat parental band loss and 74.5% and 68.4% of the rye parental band loss occurred in the F_1 hybrids (before chromosome doubling) for E–M and P–M primers, respectively. The sequence variation events that followed chromosome doubling consisted of continuous modifications that occurred at a very small rate compared with the rate of variation before chromosome doubling. However, the rate of sequence variation involving the rye parental genome was much higher in the first 5 generations following chromosome doubling than in any subsequent generation. Surprisingly, the highest rate of rye genomic variation occurring after chromosome doubling was in C_3 or later, but not in C_1. The data suggested that the cytoplasm and the degree of the relationship between the parental genomes were the key factors in determining the direction, amount, timing, and rate of genomic sequence variation occurring during intergeneric allopolyploidization.

Key words: genome evolution, sequence variation, allopolyploid, triticale, AFLP.

Résumé : Le moment et le taux d'apparition de variation génomique découlant de l'allopolyploïdisation chez le triticale (× *Triticosecale* Wittmack), un hybride intergénérique entre le blé et le seigle, ont été étudiés en examinant le polymorphisme de longueur des fragments amplifiés (AFLP). Le polymorphisme a été révélé à l'aide de deux jeux d'amorces, *Eco*RI–*Mse*I (E–M) et *Pst*I–*Mse*I (P–M), lesquels amplifient respectivement des séquences répétées et à faible nombre de copies. Les résultats montrent que l'allopolyploïdisation entraîne des variations de séquence génomique chez le triticale et qu'une grande partie de cette variation survient immédiatement à la suite de l'hybridation. Spécifiquement, environ 46,3 % et 36,2 % des pertes de fragments du blé ainsi que 74,5 % et 68,4 % des pertes de fragments du seigle sont survenues au sein de la F_1 (avant le doublement chromosomique) pour les amorces E–M et P–M respectivement. Les variations survenant après le doublement chromosomique survenaient de manière continue mais à faible fréquence par rapport à la variation se produisant avant le doublement. Le taux d'apparition de variants impliquant le génome du seigle était beaucoup plus élevé au cours des cinq premières générations suivant le doublement que dans les générations subséquentes. Étonnamment, la plus grande variation au sein du génome du seigle se produisant après le doublement chromosomique est survenue en C_3 ou ultérieurement, mais pas en C_1. Les données suggèrent que le cytoplasme et le degré de parenté entre les génomes parentaux sont les facteurs principaux déterminant la direction, l'ampleur, le moment et le taux de variation des séquences génomiques survenant lors d'allopolyploïdisation intergénérique.

Mots clés : évolution du génome, variation de séquence, allopolyploïde, triticale, AFLP.

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Introduction

Allopolyploidy has long been recognized as an evolutionary process whereby 2 or more different, but usually closely related, genomes are brought together into the same nucleus by either interspecific or intergeneric hybridization, followed by chromosome doubling. Genome-wide gene redundancy not only enables allopolyploids to tolerate more genomic variation compared with their progenitors, but also provides novel opportunities to generate functional diversifi-

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X.-F. Ma[1] and J.P. Gustafson.[2,3] Department of Agronomy, University of Missouri, Columbia, MO 65211, USA.

[1]Present address: Forage Improvement Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA.
[2]Present address: USDA–ARS, Plant Genetics Research Unit, University of Missouri, Columbia, MO 65211, USA.
[3]Corresponding author (e-mail: pgus@missouri.edu).

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cation between homoeologous genes and genomes (Adams and Wendel 2005), thus increasing their fitness in nature, which results in allopolyploids being widespread in flowering plants (Wendel 2000). In addition, recent molecular and cytological technologies have revealed many novel paleopolyploids, which have been traditionally considered to be diploids (Ma and Gustafson 2005). However, their polyploid nature has been obscured because they have evolved through extensive chromosome fusions and (or) fissions and frequent rearrangements after polyploidization. Based on these observations, Liu and Wendel (2002) speculated that there are probably no bona fide diploid species in plants. This speculation has been supported, at least in cereal crops, by an ancient polyploidization event that occurred about 70 million years ago, before the major cereal crops diverged from one another (Paterson et al. 2004).

These novel recognitions raised an interesting question: how do polyploid genomes evolve after their formation? Recently, researchers have addressed this issue based on newly formed allopolyploids in a few species, such as *Arabidopsis*, *Brassica*, cotton (*Gossypium* spp.), wheat (*Triticum* spp.), and triticale (× *Triticosecale* Wittmack) (Ma and Gustafson 2005). Newly synthesized allopolyploids provide an opportunity to investigate early genetic and epigenetic events, which cannot be revealed in natural allopolyploids (Song et al. 1995). It has been suggested that, except for chromosome recombination, the evolution of allopolyploids is also accompanied by other genetic and epigenetic modifications, which lead to genetic diploidization, a process that establishes a diploid-like behavior in polyploid genomes (Ma and Gustafson 2005).

A typical observation of genetic changes in newly synthesized allopolyploids is sequence elimination. Studies in the wheat group (*Aegilops–Triticum* complex) have indicated that sequence elimination is a rapid, directional and highly reproducible event. It has been speculated that the elimination of specific sequences augmented the differentiation of homoeologous chromosomes at the polyploid level, thus facilitating the diploidization process of polyploid wheat (Ozkan et al. 2002; Levy and Feldman 2002, 2004; Feldman and Levy 2005). In addition, polyploidization-induced rapid epigenetic modifications and changes in gene expression have been reported in wheat (Kashkush et al. 2002, 2003; He et al. 2003). Similar genetic and epigenetic phenomena have been shown in other species (Chen and Ni 2006).

Though many studies have been conducted, the genetic mechanisms contributing to allopolyploid speciation remain elusive. Thus, more studies are needed to fully understand the genomic evolution of allopolyploids. Here we present a study that was undertaken in the new cereal hybrid triticale.

Triticale is a cereal originating from the chromosome-doubled hybrid of various wheat species (AA, AABB, and AABBDD) and rye (*Secale cereale* L., RR). Since the first attempt to produce an artificial hybrid (Wilson 1876) and the first recognition of large scale natural hybridization between wheat and rye (Meister 1921), thousands of triticales have been synthesized in a variety of ploidy levels and genome combinations, such as tetraploid AARR, hexaploid AABBRR, and octoploid AABBDDRR. Because of its recent origin and the availability of its parents, the triticale group has recently been used for studying the genome evolutionary behavior of allopolyploids (Voylokov and Tikhenko 2002; Ma et al. 2002, 2004). Compared with the other allopolyploid species studied, the parental relationship of triticale is much more distant, which could result in a genomic response to allopolyploidization that is more extensive than it is for other hybrids, such as *Arabidopsis, Brassica*, cotton, and wheat. The results of previous studies in triticales using amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) analyses indicated considerable genome variation, with most of the alterations occurring in the rye parental genome (Ma et al. 2002, 2004). The data also suggested that sequence elimination might be a force causing genome variation in triticale, since the frequency of losing parental bands was much higher than that of gaining novel bands, regardless of the circumstances (Ma et al. 2004). These results raised an interesting question: how fast had these genomic changes occurred in the various triticales following speciation? In the present study, this issue was addressed by studying the timing and rate of genomic variation with early generation triticale lines. The early generation materials were obtained by recreating the 4 triticales used in previous studies (Ma et al. 2002, 2004), in which plants grown from the same seed sources were used. All together, the materials used in this study included 4 sets of wheat and rye parents, their F_1 hybrids, the first 3 or 5 allopolyploid generations after chromosome doubling, and the previously synthesized triticales, which are at least 15 or 35 generations old.

The objective of this study was to investigate the timing and rate of genome variation at a genome-wide level. For this reason, AFLP analysis was used because of its wide coverage of genomic sequences. For the same reason, we also used 2 kinds of primer combinations, *Eco*RI–*Mse*I and *Pst*I–*Mse*I primers, which primarily amplify repetitive and low-copy sequences, respectively, as a result of their relative differences in sensitivity to cytosine methylation (Ma et al. 2004). In the previous study, these 2 kinds of primers showed different degrees of genomic DNA variation between repetitive and low-copy sequences (Ma et al. 2004). Thus, the same strategy was also used in the present study to investigate the timing and rate of genome changes in both repetitive and low-copy sequences in wheat–rye hybrids of various ages.

Materials and methods

Plant materials and DNA isolation

In addition to the 2 previously synthesized hexaploid triticales (at least 15 generations old), 2 octoploid triticales (at least 35 generations old), and their exact wheat and rye progenitors (Ma et al. 2004), the present study also included the corresponding wheat–rye F_1 hybrids and the first 3 or 5 generations of the 4 triticales after chromosome doubling (Table 1). The F_1 hybrids and the new triticale lines were obtained by resynthesis with the appropriate wheat and rye parents followed by chromosome doubling with a 0.1% colchicine treatment. All parents were expected to be near homozygous as a result of many years of selfing. The seeds used for crosses were obtained from the original wheat and rye inbred seed sources used to create the old hybrids (Ma et al. 2004). For each triticale group, all progeny originated

Table 1. Plant materials and genomic constituents used in this study.

Cross	Wheat variety (genome)	Rye variety (genome)	Hybrid and triticale
CS×I	'Chinese Spring' (AABBDD)	'Imperial' (RR)	F_1, C_1, C_2, C_3, C_4, C_5, $T_{>35}$
H×K	'Holdfast' (AABBDD)	'King II' (RR)	F_1, C_1, C_2, C_3, $T_{>35}$
C×S	'Cocorit 71' (AABB)	'Snoopy' (RR)	F_1, C_1, C_2, C_3, $T_{>15}$
C×U	'Cocorit 71' (AABB)	'UC90' (RR)	F_1, C_1, C_2, C_3, $T_{>15}$

Note: F_1, hybrid of the corresponding wheat and rye; C_1–C_3 or C_5, the first 3 or 5 generations following chromosome doubling of the corresponding F_1 hybrid; T, the previously synthesized counterparts (at least 15 generations old for the 2 hexaploid triticales and 35 generations old for the 2 octoploid triticales).

from a single doubled haploid (C_0). Chromosome numbers and constitutions were determined using C banding in the first generation (C_1) of the triticales, and only those having the expected euploid chromosome complement were used. Each of the newly created triticales was planted and selfed in a greenhouse up to the C_3 or C_5 generation. Two more generations were obtained from the CS×I family than from the others because the CS×I family grew faster and did not need vernalization. Three sister lines from each generation were used for AFLP fingerprint analyses. Young leaf tissue was harvested, freeze dried, and stored at –20 °C until all materials were available for this study. Genomic DNA was isolated from dried leaves using a modified CTAB method (Saghai Maroof et al. 1984).

AFLP analysis

The original protocol of Vos et al. (1995) was followed with some modifications. The digestion of genomic DNA (500 ng) with *Eco*RI or *Pst*I and *Mse*I restriction enzymes and ligation were performed separately in 1× One-Phor-All buffer (Amersham Pharmacia Biotech, Piscataway, N.J.) in a final volume of 60 µL. For pre-amplification, 2.5 µL of the digestion–ligation reaction was used in a 20 µL reaction volume containing primers (75 ng) with 1 selective nucleotide, 0.2 mmol/L of each dNTP, 1 U of *Taq* polymerase, and 1× *Taq* polymerase buffer containing 1.5 mmol/L $MgCl_2$. *Eco*RI or *Pst*I primers were 5′ end labeled with [Y-^{33}P]ATP using 0.1 U of T4 PNK (polynucleotide kinase) and 1× One-Phor-All buffer. The pre-amplification reaction was diluted 20-fold and 2.5 µL was used for a second amplification step containing primers with 3 selective nucleotides in a 10 µL reaction mixture containing 2.5 ng of the labeled *Eco*RI or *Pst*I primer, 15 ng of *Mse*I primer, 0.2 mmol/L of each dNTP, 0.25 U of *Taq* polymerase, and 1× *Taq* polymerase buffer containing 1.5 mmol/L $MgCl_2$. All reactions were performed in a PCR Express thermal cycler (Hybaid, Franklin, Mass.) using 96 well plates. Marker designations contained the primer combination used (abbreviated by the initial of the restriction enzyme followed by the selective nucleotides). A total of 36 AFLP primer combinations were used to analyze the CS×I family, but only 20 of them were used for the others.

Results

The timing and rates of genomic change were investigated using AFLP analyses on the newly created hybrid materials. Since the CS×I family contained 2 more generations than other triticale groups, 16 more primer combinations were used in the CS×I group (36 primers) than in the others (20 primers). As expected, *Eco*RI–*Mse*I (E–M) primers generally produced denser AFLP profiles than *Pst*I–*Mse*I (P–M) primers, as shown in Figs. 1 and 2, since E–M primers amplified more repetitive sequences than P–M primers (Ma et al. 2004). For this reason, the data were collected separately for each type of primer. In total, about 10 000 AFLP bands were scored, including over 4800 E–M bands and 5200 P–M bands. For each set of crosses, the AFLP bands were scored for the presence or absence of wheat, rye, their F_1 hybrid, and the series of triticale lines derived from them and classified into 15 different patterns (Table 2).

The data not only confirmed previous results (Ma et al. 2002, 2004) that a high degree of genomic change occurred in the wheat and rye genomes when they co-existed in a triticale background, but also distinguished the amount of genomic variation occurring before or after chromosome doubling. In particular, a considerable degree of genomic sequence changes or modifications existed in all of the wheat–rye hybrids immediately after intergeneric hybridization, and a gradual process of additional sequence changes occurred

Fig. 1. AFLP banding profiles of the 'Chinese Spring' × 'Imperial' lines when amplified with PAGA–MCCC. W, wheat; R, rye; H, F_1 hybrid; T, triticale; C_1–C_5, the first 5 generations of the triticale after chromosome doubling. The nos. from 1 to 3 in each generation represent 3 sister lines in the corresponding generation. The 3 sister lines in the first generation (C_1) were all derived from a single doubled haploid (C_0), and the 3 sister lines in each of the subsequent generation were all derived from the first plant (No. 1) of the previous generation. Arrows indicate those rye bands that are absent in some of the triticale lines.

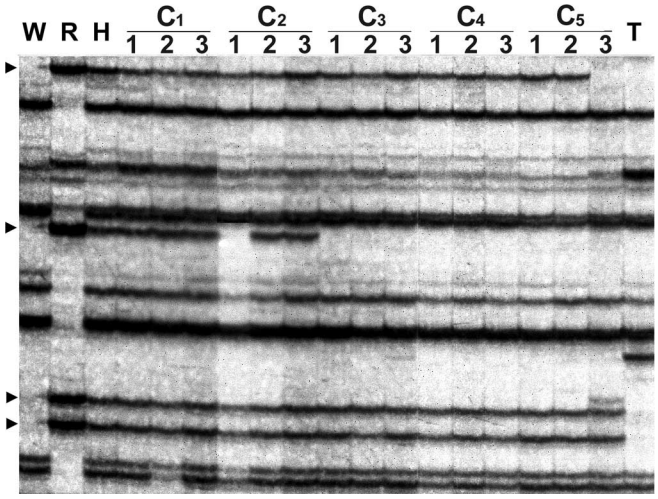
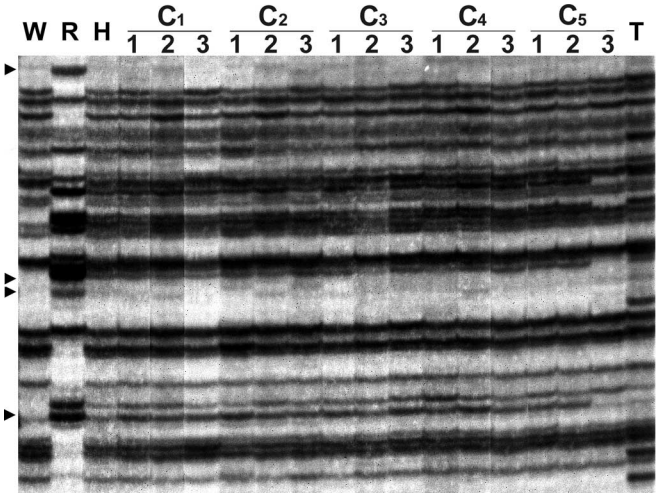


Fig. 2. AFLP banding profiles of the 'Chinese Spring' × 'Imperial' lines when amplified with ECCA–MCGA. W, wheat; R, rye; H, F_1 hybrid; T, triticale; C_1–C_5, the first 5 generations of the triticale after chromosome doubling. The nos. from 1 to 3 in each generation represent 3 sister lines in the corresponding generation. The 3 sister lines in the first generation (C_1) were all derived from a single doubled haploid (C_0), and the 3 sister lines in each of the subsequent generation were all derived from the first plant (No. 1) of the previous generation. Arrows indicate those rye bands that are absent in the F_1 hybrid and (or) some of the triticale lines.



in the early generations of the triticales following chromosome doubling.

Immediate, dramatic responses to intergeneric hybridization

Since band loss contributed to most of the genomic variation observed, only data on the numbers of lost bands in triticale shown in Table 2 were collected in Table 3 based on their presence or absence in the hybrids relative to their wheat and rye parents. For each primer combination, 3 major banding classes were formed based on their parental types, including + –, – +, and + +, for wheat and rye parents, respectively. The proportions of bands that were present and absent for the F_1 hybrids were calculated for each class. The data in Table 3 shows the number and percentage of bands lost in the F_1 hybrids compared with that lost after chromosome doubling. For each parental type, the data in the upper row represents the number and percentage of bands lost after chromosome doubling, and the data in the second row represents the number and percentage of bands lost before chromosome doubling.

In the hybrids, the variation level of wheat parental bands was different from that of rye parental bands in all cases. For wheat parental bands (parental type +, –), the majority of the bands lost in triticale were still present in the F_1 hybrids. Except for C×U when E–M primers were used, the proportion of wheat parental band loss was about 30%–40% in the F_1 hybrids in all other cases, i.e., 40.0%, 43.5%, and 38.1% for CS×I, H×K, and C×S, respectively, when E–M primers were used, and 28.6%, 40.0%, 39.4%, and 40.0% for CS×I, H×K, C×S, and C×U, respectively, when P–M primers were used (Table 3). The data suggested that 60%–70% of the wheat banding loss occurred after chromosome doubling. The proportions of C×U when amplified with E–M primers were very different from those of the other materials, showing that 62.5% of the wheat parental band loss had already occurred in the F_1 hybrids. This suggested that rates of genomic variation can be very different for different parental backgrounds. On average, the current data indicated that, for the E–M and P–M primers, 46.3% and 36.2%, respectively, of the wheat parental band loss occurred before chromosome doubling, and 53.7% and 63.8% occurred after chromosome doubling (Table 3).

However, for rye parental bands (parental type – +), most of the band loss events occurred in the F_1 hybrids, before chromosome doubling (Table 3). For each type of primer, differences in rye AFLP banding variation were not obvious among the different materials. Overall, for E–M and P–M primers,74.5% and 68.4% of the rye parental band loss, respectively, occurred in the F_1 hybrids, and only 25.5% and 31.6% occurred after chromosome doubling. The data in Table 3 also included the parental type + +, which showed the numbers of AFLP bands that were lost when present in both wheat and rye parents. Since the numbers of the parental type + + were very small, the proportions of bands lost may have been biased when they were present in both parents.

In addition, for both wheat and rye parental types (+ – and – +), the numbers of bands lost in the F_1 hybrids were higher when amplified by E–M primers than by P–M primers (46.3% vs 36.2% and 74.5% vs 68.4%, respectively), suggesting that in the F_1 hybrids, more variation occurred in repetitive sequences than that in low-copy sequences. As observed previously (Ma et al. 2004), the general trend suggested that repetitive sequences respond more quickly and extensively than low-copy sequences to intergeneric hybridization. Rapid and extensive changes in repetitive sequences may facilitate the overall compatibility between wheat and rye genomes in the F_1 hybrids and further promote the genetic diploidization process after chromosome doubling.

Gradual modifications after chromosome doubling

The rates of genomic variation were also recorded following chromosome doubling, based on the early generations of the recreated triticales. The results revealed only a few changes in the wheat parental banding patterns during the first 3–5 allopolyploid generations, thus indicating that the occurrence of further AFLP banding variation in the wheat parental genome, following chromosome doubling, was a slow process. Since the numbers of bands for this category were very small, the variation in the wheat parental band after chromosome doubling was not further analyzed. However, additional variation in rye parental bands was detectable in the early generation triticale lines following chromosome doubling (Table 4). The data supported the overall observation that most of the wheat parental bands remained present in triticale, but a large percentage of the rye parental bands was absent.

Since 3 sister lines were used in each generation of the recreated triticales, a band was scored as absent if it showed polymorphism among 3 sister lines of a generation, i.e., the band was missing in at least 1 of the 3 sister lines, thus slightly over-estimating the number of bands lost in each generation. However, the 3 sister lines in each generation of

Table 2. Numbers of AFLP fragments of each banding pattern.

Primer	W	R	H	T	CS×I	H×K	C×S	C×U	Total
*Eco*RI–*Mse*I									
	+	–	+	+	834	420	375	354	1983
	+	–	+	–	156	65	156	93	470
	+	–	–	+	0	9	19	16	44
	+	–	–	–	104	50	96	155	405
	–	+	+	+	173	79	116	91	459
	–	+	+	–	69	29	46	35	179
	–	+	–	+	0	17	35	14	66
	–	+	–	–	161	124	136	102	523
	+	+	+	+	109	59	82	77	327
	+	+	+	–	8	4	21	36	69
	+	+	–	+	0	2	2	2	6
	+	+	–	–	5	3	5	16	29
	–	–	+	+	0	12	5	10	27
	–	–	+	–	0	5	12	18	35
	–	–	–	+	153	16	38	19	226
	Total				1772	894	1144	1038	4848
*Pst*I–*Mse*I									
	+	–	+	+	876	596	460	377	2309
	+	–	+	–	45	12	40	30	127
	+	–	–	+	0	19	17	10	46
	+	–	–	–	18	8	26	20	72
	–	+	+	+	224	104	154	106	588
	–	+	+	–	138	45	81	63	327
	–	+	–	+	7	29	51	16	103
	–	+	–	–	284	165	144	116	709
	+	+	+	+	163	233	146	93	635
	+	+	+	–	5	4	8	6	23
	+	+	–	+	0	1	1	0	2
	+	+	–	–	4	7	4	2	17
	–	–	+	+	0	19	9	9	37
	–	–	+	–	0	35	16	27	78
	–	–	–	+	95	18	34	31	178
	Total				1859	1295	1191	906	5251

Note: W, wheat; R, rye; H, F_1 hybrid; T, old triticale line (at least 35 generations old for CS×I and H×K, and 15 generations old for C×S and C×U); CS×I, 'Chinese Spring' × 'Imperial'; H×K, 'Holdfast' × 'King II'; C×S, 'Cocorit 71' × 'Snoopy'; C×U, 'Cocorit 71' × 'UC90'; +, present; –, absent.

a triticale group usually showed a similar pattern of band presence or absence, indicating that the genomic variation observed in the triticales was reproducible.

The results indicated that loss of rye parental bands after chromosome doubling was a continuous process, but occurred at a very low rate (Table 4). Since there were 2 more generations available in the CS×I triticale family, the patterns of rye parental band loss in CS×I were more evident than in the other triticales. It was also obvious that the patterns of loss were similar for the 2 kinds of primers. The proportions of lost bands increased in the first 5 generations of CS×I, reaching the highest level at the C_5 generation, with 7.4% for E–M primers and 8.8% for P–M primers. As a result, the total accumulated proportions of lost bands accounted for 17.0% and 17.8% for the first 5 generations of the CS×I triticales when E–M and P–M primers were used, respectively. This suggested that most of the rye genome variation occurring after chromosome doubling happened in the first 5 generations of the CS×I triticale family. The percentage of the total loss (13.0% for E–M primers and 14.9% for P–M primers) observed after C_5 in the CS×I family occurred during a time period covering at least 30 generations, implying that the amount of genomic variation following chromosome doubling reached its highest point at C_5, or soon after, and then leveled off.

Similar results were also observed in the other 3 sets of triticales, in which the tendency to lose bands in the first 3 generations agreed with that observed for the first 3 generations of the CS×I triticales (Table 4). The variation in the rye genome over generations occurred in a similar manner in all the triticales studied. A notable observation was that the level of rye genome alteration increased from C_1 to C_3 (or C_5). This finding was inconsistent with our speculation that the highest amount of genomic variation after chromosome doubling was expected to be in the first allopolyploid generation (C_1).

The data from this study and our previous studies (Ma et al. 2002, 2004) indicated the following: (*i*) wide hybridization and allopolyploidization (genome doubling) induced a considerable degree of genomic variation in triticale;

Table 3. Numbers and percentages of AFLP fragments lost before and after chromosome doubling.

					CS×I		H×K		C×S		C×U		Total	
Primer	W	R	H	T	No.	%	No.	%	No.	%	No.	%	No.	%
*Eco*RI–*Mse*I														
Band class 1			+		156	60.0	65	56.5	156	61.9	93	37.5	470	53.7
	+	–		–										
			–		104	40.0	50	43.5	96	38.1	155	62.5	405	46.3
Band class 2			+		69	30.0	29	19.0	46	25.3	35	25.5	179	25.5
	–	+		–										
			–		161	70.0	124	81.0	136	74.7	102	74.5	523	74.5
Band class 3			+		8	61.5	4	57.1	21	80.8	36	69.2	69	70.4
	+	+		–										
			–		5	38.5	3	42.9	5	19.2	16	30.8	29	29.6
*Pst*I–*Mse*I														
Band class 1			+		45	71.4	12	60.0	40	60.6	30	60.0	127	63.8
	+	–		–										
			–		18	28.6	8	40.0	26	39.4	20	40.0	72	36.2
Band class 2			+		138	32.7	45	21.4	81	36.0	63	35.2	327	31.6
	–	+		–										
			–		284	67.3	165	78.6	144	64.0	116	64.8	709	68.4
Band class 3			+		5	55.6	4	36.4	8	66.7	6	75.0	23	57.5
	+	+		–										
			–		4	44.4	7	63.6	4	33.3	2	25.0	17	42.5

Note: For each band class, the data in the upper row are the numbers and percentages of bands lost after chromosome doubling and the data in the bottom row are the numbers and percentages of bands lost before chromosome doubling. W, wheat; R, rye; H, F_1 hybrid; T, triticale (at least 35 generations old for CS×I and H×K, and 15 generations old for C×S and C×U); CS×I, 'Chinese Spring' × 'Imperial'; H×K, 'Holdfast' × 'King II'; C×S, 'Cocorit 71' × 'Snoopy'; C×U, 'Cocorit 71' × 'UC90'; +, present; –, absent. The percentages refer to the proportions within each parental type.

Table 4. Numbers and percentages of rye parental bands lost in each set of the materials.

		H		C_1		C_2		C_3		C_4		C_5		T		
Cross	Primer	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%[a]	Total No.
CS×I	E–M	161	70.0	1	0.4	4	1.7	9	3.9	8	3.5	17	7.4	30	13.0	230
	P–M	284	67.3	0	0.0	2	0.5	17	4.0	19	4.5	37	8.8	63	14.9	422
H×K	E–M	124	81.0	2	1.3	2	1.3	6	3.9					19	12.4	153
	P–M	165	78.6	1	0.5	1	0.5	8	3.8					35	16.7	210
C×S	E-M	136	74.7	4	2.2	8	4.4	10	5.5					24	13.2	182
	P–M	144	64.0	5	2.2	9	4.0	12	5.3					55	24.4	225
C×U	E–M	102	74.5	1	0.7	3	2.2	4	2.9					27	19.7	137
	P–M	116	64.8	0	0.0	3	1.7	10	5.6					50	27.9	179

Note: E–M, *Eco*RI–*Mse*I; P–M, *Pst*I–*Mse*I; H, F_1 hybrid; C_1–C_5, the first 5 generations of allopolyploid triticale lines; T, old triticale line (at least 35 generations old for CS×I and H×K, and 15 generations old for C×S and C×U). CS×I, 'Chinese Spring' × 'Imperial'; H×K, 'Holdfast' × 'King II'; C×S, 'Cocorit 71' × 'Snoopy'; C×U, 'Cocorit 71' × 'UC90'; The percentages refer to the proportions of rye parental band loss in the corresponding generations (H to C_3 or C_5) compared with the total rye band loss.

[a]The accumulated proportion of rye band loss for all subsequent generations after C_3 or C_5 compared with the total rye band loss.

(*ii*) most of the variation that occurred in the rye parental genome was triggered by intergeneric hybridization rather than by chromosome doubling; (*iii*) genomic variation after chromosome doubling was a slow, continuous process; and (*iv*) for the rye parental genome, the process showed a relatively high rate of variation in the first 5 generations following chromosome doubling. Compared with other allopolyploid species studied, the current study in triticales provided the most information regarding the rate of genomic sequence alteration before and after chromosome doubling during allopolyploidization, which greatly extends our knowledge and provides a novel insight into the evolutionary behavior of allopolyploid genomes.

Discussion

Timing and rate of genomic variation

The current data not only confirmed earlier observations (Ma et al. 2002, 2004) that sequence variations occurred during allopolyploidization in the wheat–rye hybrid triticale, but also demonstrated the amount of genomic change that

occurred after crossing, in both the F_1 and following colchicine treatment, in the amphidiploid. In all of the materials studied, the majority of wheat genome changes occurred after chromosome doubling; however, in the rye genome, most of the sequence variation events occurred before chromosome doubling. The results add a significant amount of new information to the observations from earlier studies involving wheat, in which the timing and rate of sequence elimination in 2 newly synthesized allopolyploid wheats were studied using AFLP analysis (Shaked et al. 2001). Those results indicated that, in an *Aegilops sharonensis* × *Aegilops umbellulata* hybrid, 14% of the loci from *A. sharonensis* were eliminated, compared with only 0.5% from *A. umbellulata*, and that most of the changes occurred in the F_1 hybrid. In contrast, in another cross, *Aegilops longissima* × *Triticum urartu,* sequence elimination was more frequent after chromosome doubling (Shaked et al. 2001), which suggested that the timing and rate of sequence variation could be very different among various wheat allopolyploids. However, the differences in the timing and rate of sequence variation were not as evident in different triticales compared with the results observed in wheat. These differences might be due to the fact that the allopolyploid triticale underwent a much higher degree of sequence variation than the allopolyploid wheat, and that the greater level of sequence variation in triticale may have obscured any influences caused by differences in the wheat and rye parental backgrounds. In addition, the current data displayed the timing and rate of genomic sequence variation after chromosome doubling, whereas the same information was not seen in the wheat study because only the first-generation allopolyploid wheats were used (Shaked et al. 2001). In another wheat study, the timing and rate of sequence elimination using up to 3 generations following allopolyploidization were also investigated (Ozkan et al. 2001), but this study only involved 5 chromosome-specific sequences (CSSs) and 3 genome-specific sequences (GSSs). The results indicated that elimination of GSSs was already initiated in F_1 plants, whereas the elimination of CSSs started in the first allopolyploid generation, although, in both cases, the elimination process was completed by the second or third allopolyploid generation. One difference between wheat and triticale is that, in triticale, the rate of sequence variation in the F_1 hybrids was much higher than that in allopolyploids in all cases, regardless of the materials or primers used, while this tendency was not true for the wheat group (Shaked et al. 2001; Ozkan et al. 2001). Another major difference was that, in triticale, genomic changes were highly skewed to 1 parent, i.e., rye, and a majority of the rye parental banding loss occurred in a single generation (F_1), with a proportion, on average, as high as 74.5% for E–M primers and 68.4% for P–M primers. The mechanisms of this rapid, selective sequence modification/change in 1 parent are not clear and deserve further discussion.

Potential causes of genomic variation

Since all parental materials were strictly inbred lines over many years of self-crossing, any genomic changes should be directly related to the genomic response to allopolyploidization. One explanation of the highly directed genomic changes involves the wheat cytoplasmic background, which was common in all of the triticales studied. Since rye–wheat hybrids (i.e., rye used as the maternal parent) are extremely rare and unstable, all the triticales used were derived from wheat–rye hybridization, and had inherited wheat cytoplasm. The nuclear–cytoplasmic interaction hypothesis suggests that the paternal genome may be more vulnerable to change in a newly formed hybrid because it is exposed to the "hostile" environment of maternal cytoplasm (Gill 1991). Cytoplasm-mediated directional sequence changes were also studied using RFLP analysis in *Brassica*, with 1 pair of reciprocal interspecific crosses between 2 diploid species, *Brassica rapa* (the A genome) and *Brassica nigra* (the B genome) (Song et al. 1995). The synthesized tetraploids were analogous to the natural polyploid *Brassica juncea*. The synthesized AB tetraploid contained the A genome cytoplasm, and in those plants, large directional changes were observed in the paternally donated nuclear genome (the B genome). Similar directional changes were also shown in the AB tetraploid's natural counterpart, *B. juncea*, which also contained the A cytoplasm. Other data also indicated that *B. juncea* was more similar to its maternal parent (the A genome donor) than to its paternal parent (the B genome donor) (Song et al. 1988). Directional changes were also observed in the BA tetraploid, in which the paternal genome (A genome) showed more alterations than the maternally denoted genome (B genome). These results are consistent with the current findings that the genome from the male parent, i.e., rye, was involved in a rapid process of genomic sequence variation in triticale following intergeneric wide hybridization. However, an exception to Gill's hypothesis was that, in *Ae. sharonensis* × *Ae. umbellulata*, the frequency of loci elimination in the maternal genome was much higher than that in the paternal genome (Shaked et al. 2001).

The degree of the relationship between 2 parents is also speculated to be a general factor affecting the amount, and probably the direction, of genomic sequence variation in allopolyploids. In another reciprocal interspecific *Brassica* cross involving *B. rapa* (A genome) and *B. oleracea* (C genome) (Song et al. 1995), significant directional genomic changes were not observed and the overall amount of genomic change in both the AC and CA tetraploids was much lower than that in the AB and BA tetraploids discussed. This was because the genetic distance of the AC and CA tetraploids was much closer than that of the AB and BA tetraploids (Song et al. 1995). Since the genetic distance between the parents of triticale is much further apart than those of previously studied allopolyploids, including *Arabidopsis*, *Brassica*, cotton, and wheat, it is reasonable to expect greater genomic change in triticale than in other species.

In addition, the difference in ploidy levels between wheat and rye may have an added effect on the amount of genomic variation. Chromosome structural rearrangement (Ma and Gustafson 2005) and transposon activation (Liu and Wendel 2003) are 2 other commonly observed phenomena in polyploids. However, the size range of AFLP fragments commonly scored was only around 300–800 bp, which should not be affected much by large chromosome rearrangements. Methylation repatterning is another factor contributing to AFLP banding variation, but in the present study, the difference is very limited between the 2 kinds of primers, E–M and P–M, which have relatively different sensitivities to cytosine methylation. The overall results suggested that the cy-

toplasm and the degree of relationship between the nuclear genomes of wheat and rye played significant roles in determining the direction and amount of genomic change in triticale, and those properties contributed to a considerable difference in the timing and rate of genome variation in triticale compared with that in other species studied.

Nonrandom genomic changes during allopolyploidization

Nonrandom changes refer to any genomic changes directed by evolutionary forces during allopolyploidization. Nonrandom changes help shape the allopolyploid under certain physical conditions, such as genetic or environmental conditions. On a genome-wide level, investigated using AFLP analysis, it was impossible to distinguish random from nonrandom changes; however, the current data indeed indicated nonrandom or directional changes in triticale.

This tendency could be seen from the AFLP banding profiles, in which the banding patterns of the subsequent generations (e.g., C_5 or C_3) were closer to their corresponding previously created older triticales than to the first generations (C_1) in all the materials studied, regardless of the ploidy levels or primers used, as exemplified in Fig. 1. Though random sequence modifications/changes could occur during the evolutionary process, the data indicated that at least some of the alterations were not random, but directional.

Nonrandom genome changes could involve any genomic variation that would benefit the genome diploidization process following the initial establishment of an allopolyploid. Except for structural rearrangement and epigenetic modifications, the gain and (or) loss of bands or sequences has also been reported (Ma and Gustafson 2005). Notably, studies on the C values of various polyploids have indicated that genome downsizing, or loss of DNA, might be a widespread phenomenon following polyploid formation (Leitch and Bennett 2004). It is plausible to speculate that sequence elimination could be one of the major forces shaping allopolyploids (Leitch and Bennett 2004). Low-copy sequence elimination has been well studied in newly synthesized wheat allopolyploids (Feldman and Levy 2005), and a similar phenomenon has also been seen in repetitive sequences. Recent studies in wheat (Han et al. 2005) and tobacco (Skalicka et al. 2005) showed that copies of certain repeats were eliminated rapidly and preferentially in a genome-specific manner. The elimination process was repeated and continuously targeted on the same sequence repeat in several consecutive generations (Han et al. 2005). The reproducibility of the elimination of the same repeat among different individuals derived from a single cross (Skalicka et al. 2005), or even among different allopolyploids synthesized from different parental species (Han et al. 2005), has also been observed. More importantly, at least some (Skalicka et al. 2005) or all (Han et al. 2005) of the genome changes showed concordance with changes that presumably occurred during the evolution of their natural counterparts, indicating nonrandom, selective elimination of the repeats.

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